Supplementary Table 1. List of antibodies used for staining and perturbation of adhesion function of integrins.

Antigen	Species, clonality, labeling	Isotype	Clone name	Company	Catalogue number	Application	Final concentration µg/ml
Human α1 integrin	mouse monoclonal, FITC	IgG1	TS2/7	Abcam	ab34176	FACS	10
Human α2 integrin	mouse monoclonal, FITC	IgG1	AK-7	BD Biosciences	555498	FACS	5
Human α3 integrin	mouse monoclonal	IgG1	17C6	Bio-Rad	MCA1948 GA	FACS	10
Human α6 integrin	Rat monoclonal	IgG2a	GoH3	BD Biosciences	555734	FACS	10
Human αV integrin	mouse monoclonal	IgG1	272-17E6	Abcam	ab16821	FACS, Adhesion-perturbing	10
Human β1 integrin	mouse monoclonal	IgG1	4B4	Bechman Coulter	6603113	FACS, IF, Adhesion-perturbing	10 15
Human β3 integrin	mouse monoclonal	IgG1	Y2/51	Bio-Rad	MCA2588 GA	FACS	10
Human β4 integrin	mouse monoclonal	IgG1	ASC-3	Abcam	ab78267	FACS	10
Human Collagen IV	Rabbit polyclonal	IgG1	–	Sigma	PA1-28534	IF	5
Mouse laminin	Rabbit polyclonal	IgG	–	Sigma	L9393	IF	7
Human vimentin	Rabbit monoclonal	IgG	SP20	Thermo Scientific	MA5-14564	IF	1:250
Chicken paxillin	Mouse monoclonal	IgG1	165/ Paxillin	BD Biosciences	610619	IF	10
Isotype control	Mouse monoclonal	IgG1	MOPC-21	BD Biosciences	555746	FACS, IF, Adhesion-perturbing	10 10-15
Isotype control	Rat monoclonal	IgG2a	R35-95	BD Biosciences	553927	FACS, IF, Adhesion-perturbing	10
Isotype control, FITC	Mouse monoclonal	IgG1	MOPC-21	BD Biosciences	551955	FACS	10

Supplementary Table 2. Proteins deposited by mouse astrocytes on cell culture dish identified by mass-spectrometry. Details are described in Materials and Methods section.

Click here to Download Table S2

Supplementary Table 3. Proteins deposited by human astrocytes on cell culture dish identified by mass-spectrometry. Details are described in Materials and Methods section.

Click here to Download Table S3

Supplementary information

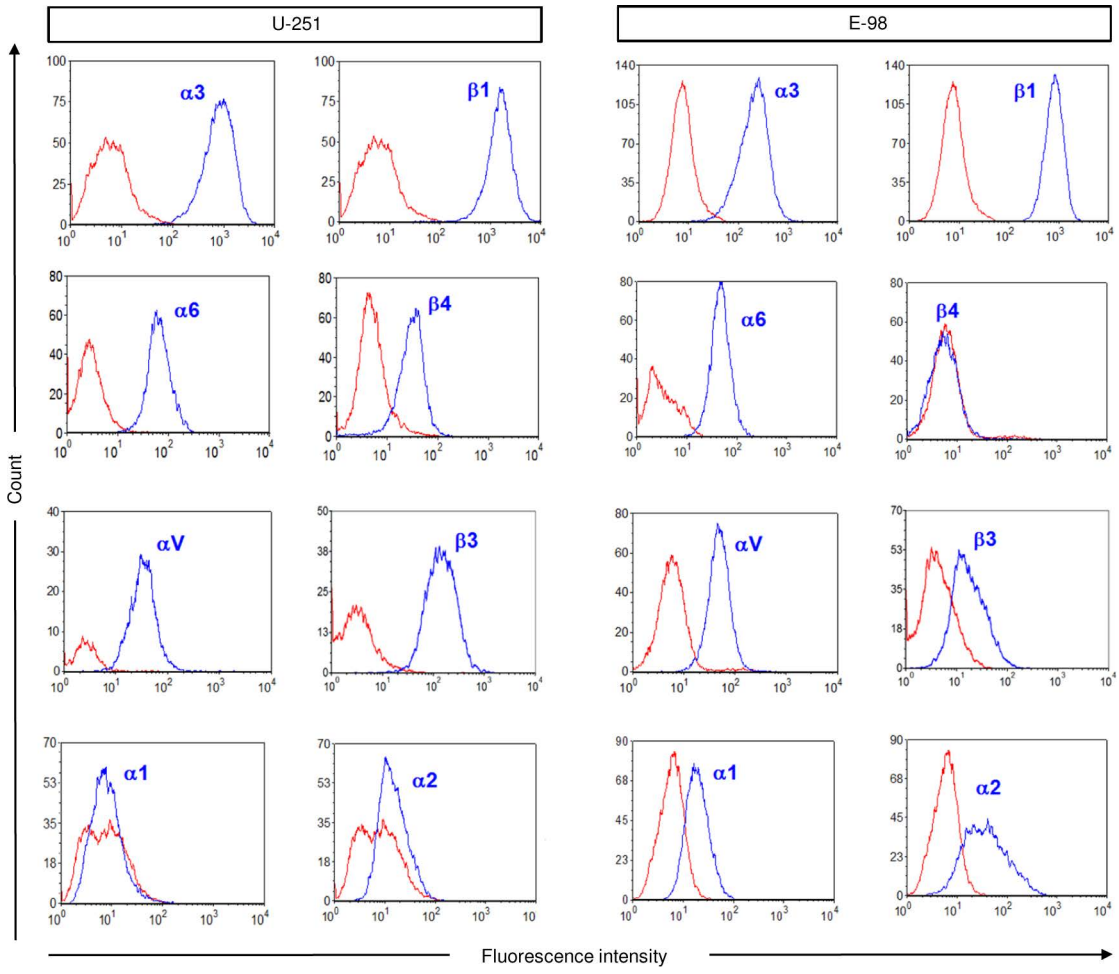
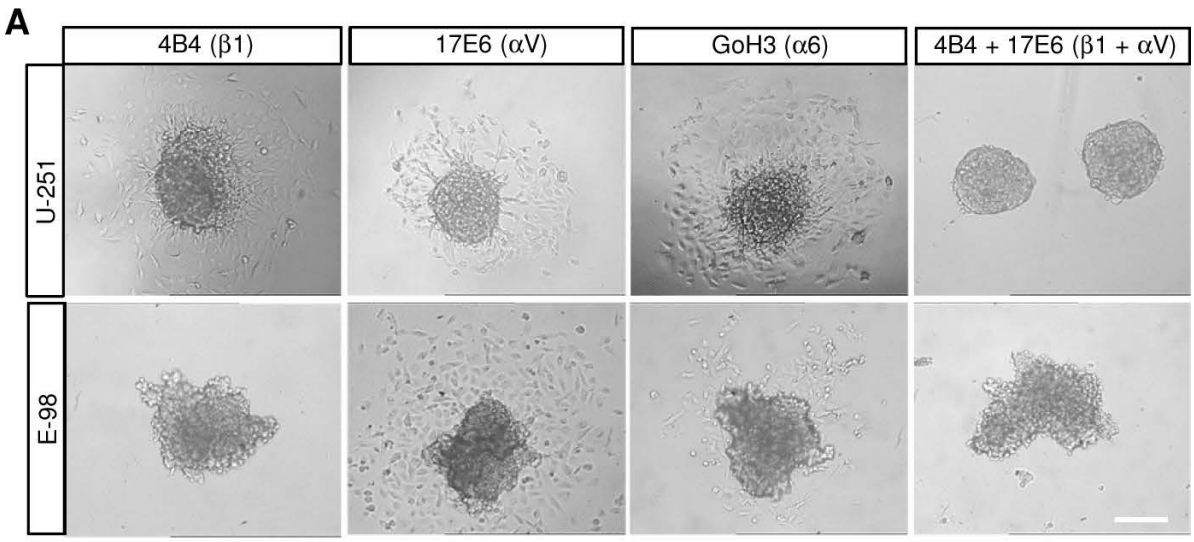
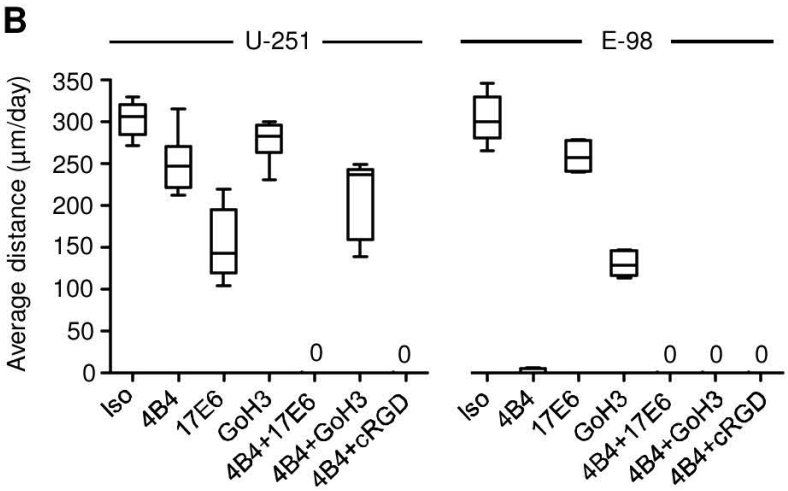
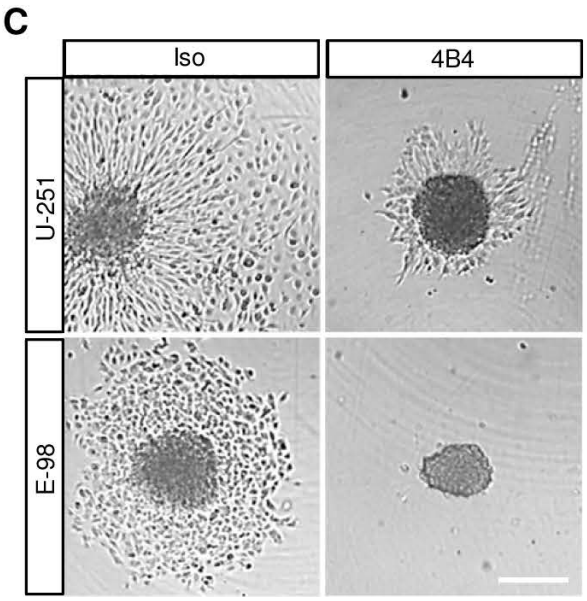


Fig. S1. Expression of integrin subunits in U-251 and E-98 cells measured with flow cytometry.







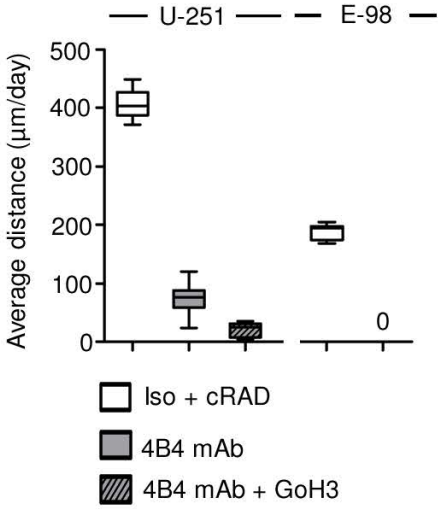
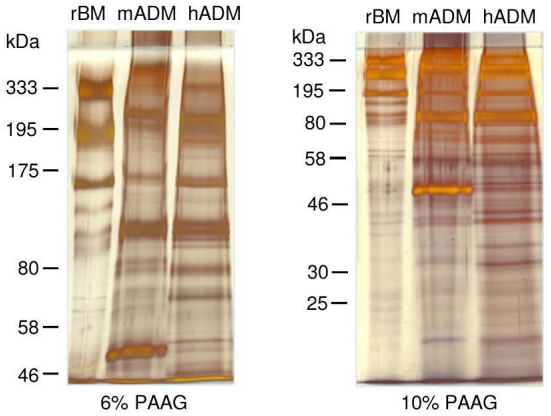


Fig. S2. Migration of glioma cells on rBM coated surface and in hyaluronan-rBM interface is dependent on β1, αV and α6 integrin subunits. (A) Radial migration of U-251 and E-98 cells from spheroids after 24 h on plastic surface coated with rBM molecules (Matrigel) in media with control isotype IgGs or with adhesion-perturbing anti-integrin mAbs 4B4 (β1), 17E6 (αV), GoH3 (α6) combined with cRGDfV peptide inhibiting RGD-binding integrins (α5, αV). (B) Average radial migration distance of U-251 and E-98 cells from the spheroid margin. Data represent 10-13 (U-251) and 10-11 (E-98) spheroids per condition from 2 independent experiments. Values display the median (black line), 25/75 percentiles (boxes) and maximum/minimum (whiskers). (C) Migration of U-251 and E-98 cells from spheroids along hyaluronan-rBM interface in media with control isotype IgGs or with adhesion-perturbing anti β1 integrin mAb 4B4 or anti α6 mAb GoH3. (D) Average migration distance of U-251 and E-98 cells along hyaluronan-rBM interface after 24 h. Data represent 10-14 (U-251) and 10-11 (E-98) spheroids per condition from 2 independent experiments. Values display the median (black line), 25/75 percentiles (boxes) and maximum/minimum (whiskers). Scale bars, 200 μm.

A



B

Matrix proteins identified both in mouse and human astrocyte matrices
nidogen-1
laminin subunit alpha-1
laminin subunit beta-1
laminin subunit gamma-1
perlecan
fibronectin
vitronectin *
collagen alpha-1(XVIII) *
laminin subunit alpha-5 *
* Proteins present in Matrigel in trace amounts

C

Proteins deposited by mouse astrocytes

PROTEIN	emPAI Sample 1	NR-PEPS Sample 1
serine protease HTRA1 precursor [Mus musculus]	2.26	17
tubulointerstitial nephritis antigen-like precursor [Mus musculus]	0.60	7
nidogen-1 precursor [Mus musculus]	0.54	16
cathelicidin-7 precursor [Bos taurus]	0.47	2
keratin 9 [Homo sapiens]	0.40	6
plasminogen activator inhibitor 1 precursor [Mus musculus]	0.39	6
vimentin [Mus musculus]	0.36	7
lysyl oxidase homolog 2 precursor [Mus musculus]	0.31	7
alpha-2-HS-glycoprotein precursor [Bos taurus]	0.28	3
keratin, type I cytoskeletal 14 [Bos taurus]	0.26	5
nidogen-1 precursor [Bos taurus]	0.25	10
PREDICTED: hemoglobin subunit beta-like [Bos taurus]	0.25	2
PREDICTED: keratin, type I cytoskeletal 10-like [Bos taurus]	0.23	4
actin, cytoplasmic 1 [Mus musculus]	0.21	3
keratin, type II cytoskeletal 1 [Mus musculus]	0.20	3
keratin, type I cytoskeletal 10 [Mus musculus]	0.17	3
laminin subunit beta-1 [Mus musculus]	0.17	9
vitronectin precursor [Bos taurus]	0.17	2
fibronectin precursor [Mus musculus]	0.16	11
keratin, type II cytoskeletal 6A [Bos taurus]	0.15	3
von Willebrand factor A domain-containing protein 1 precursor [Mus musculus]	0.15	2
PREDICTED: keratin, type II cytoskeletal 1 [Bos taurus]	0.14	3
laminin subunit gamma-1 precursor [Mus musculus]	0.13	7
PREDICTED: keratin, type I cytoskeletal 25-like [Bos taurus]	0.13	2
PREDICTED: keratin, type II cytoskeletal 2 epidermal [Bos taurus]	0.12	3
PREDICTED: keratin, type II cytoskeletal 3 [Bos taurus]	0.11	2
basement membrane-specific heparan sulfate proteoglycan core protein precursor [Mus musculus]	0.11	14
laminin subunit alpha-1 precursor [Mus musculus]	0.11	11
PREDICTED: keratin, type I cytoskeletal 13 [Bos taurus]	0.11	2
keratin, type II cytoskeletal 2 epidermal [Mus musculus]	0.10	3
collagen alpha-1(XVIII) chain isoform 2 precursor [Mus musculus]	0.10	4
lysyl oxidase homolog 4 isoform 1 precursor [Mus musculus]	0.10	3
keratin, type II cytoskeletal 7 [Bos taurus]	0.09	2
A disintegrin and metalloproteinase with thrombospondin motifs 1 precursor [Mus musculus]	0.06	2
prelamin-A/C isoform A [Mus musculus]	0.03	1
laminin subunit alpha-5 precursor [Mus musculus]	0.02	3

Fig. S3. Differences in protein composition of astrocyte-deposited matrix and rBM. (A) PAAG electrophoresis (in 6% and 10% gels) of rBM, mouse (mADM) and human (hADM) astrocyte deposited matrices (silver staining). (B) Matrix proteins identified both in mouse and human astrocyte matrix and low-abundance proteins in growth factor reduced rBM (Matrigel) based on published mass-spectrometry data (Hughes et al., 2010). (C) Proteins identified by mass-spectrometry in mouse astrocyte-deposited matrix.

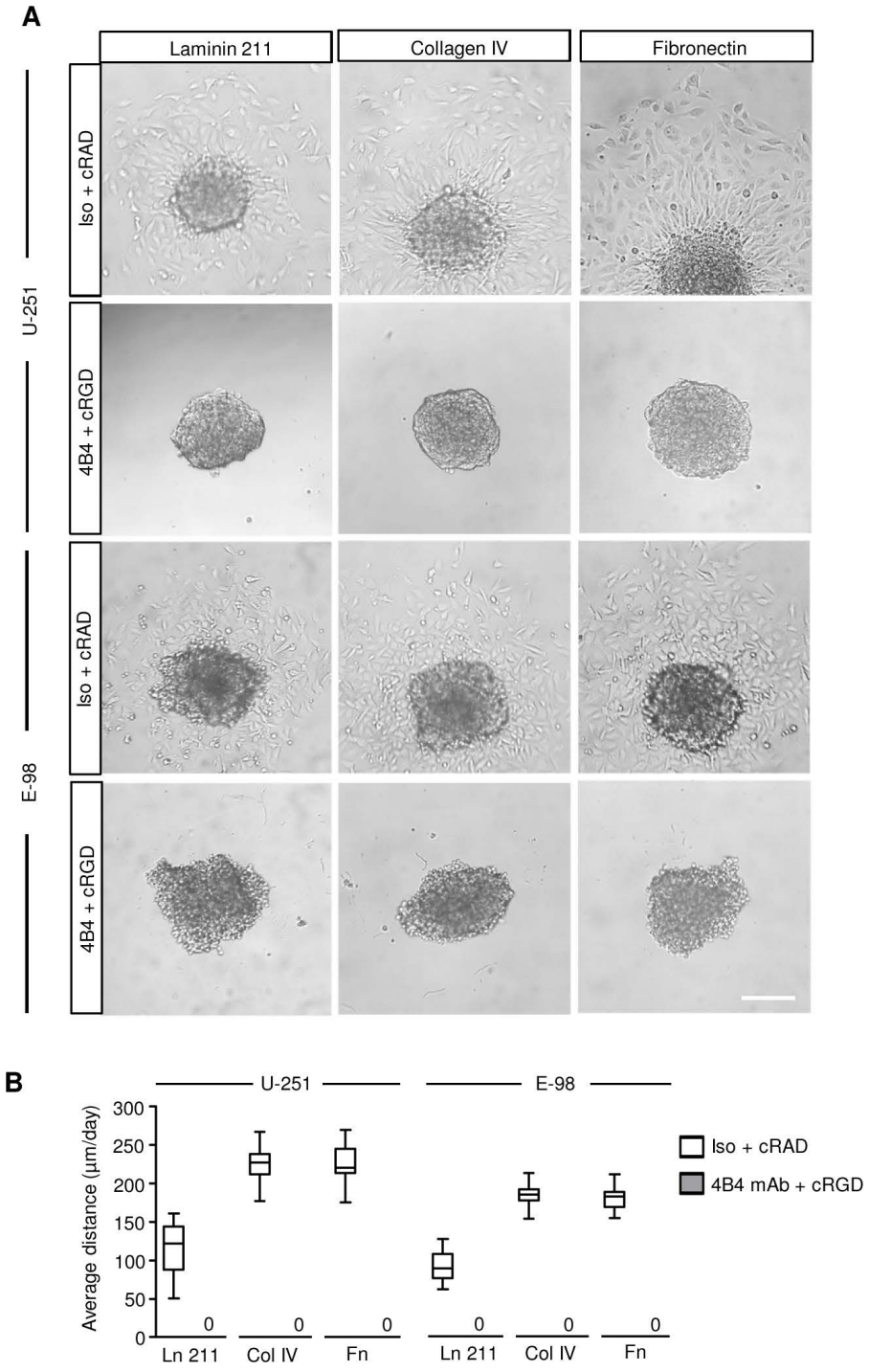


Fig. S4. Migration of glioma cells on laminin 211, collagen IV or fibronectin coated surface is dependent on β1 and αV integrin subunits. (A) Radial migration of U-251 and E-98 cells from spheroids after 24 h on plastic surface coated with the indicated matrix molecules. Isotypic IgG1 and control cRADfV peptide or adhesion-perturbing anti-β1 integrin 4B4 mAb combined with cRGDfV peptide inhibiting RGD-binding integrins (α5, αV) were added to the culture media. (B) Average migration distance of U-251 and E-98 cells on plastic surface coated with the indicated matrix molecules. Data represent 11-24 (U-251) and 16-25 (E-98) spheroids per condition from 2 independent experiments. Values display the median (black line), 25/75 percentiles (boxes) and maximum/minimum (whiskers). Scale bars, 200 µm.

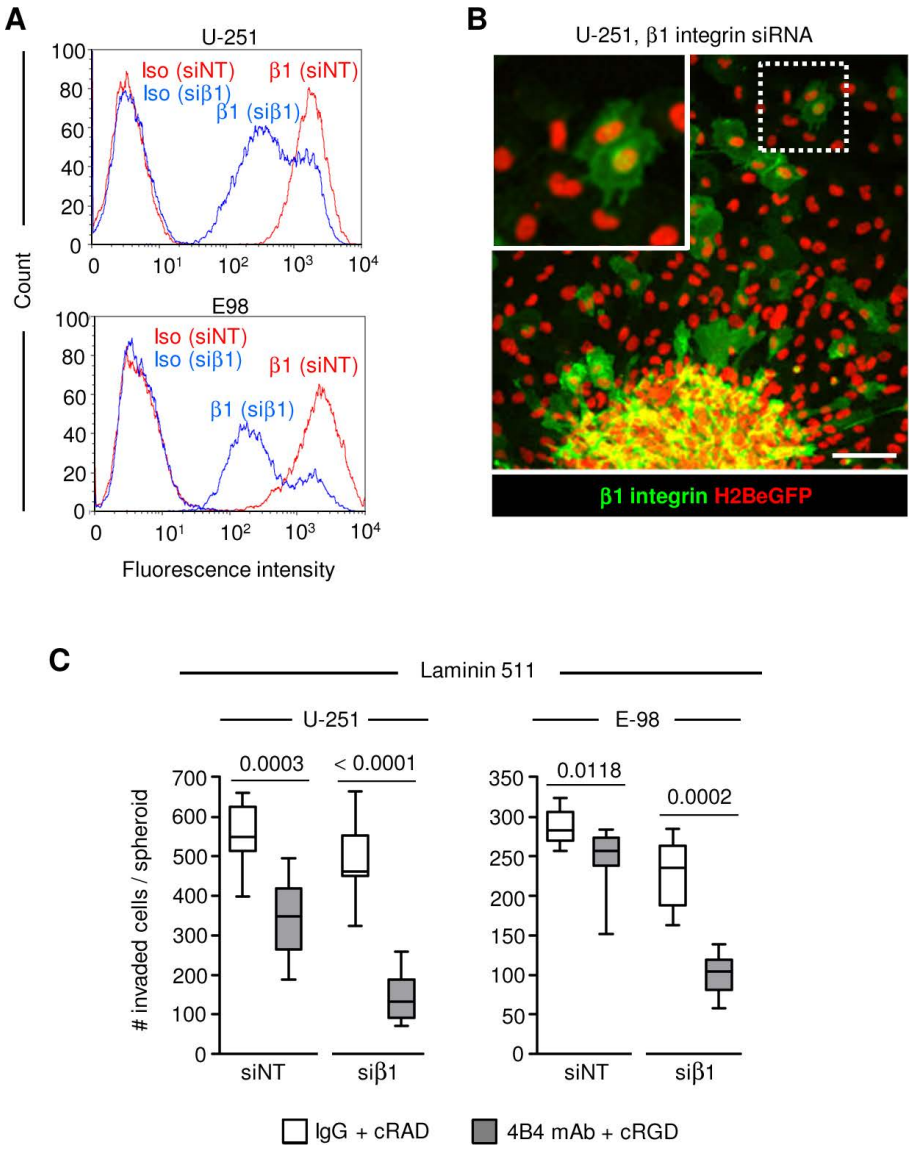


Fig. S5. β1 integrin subunits mediate glioma cell migration on laminin 511. Expression of β1 integrin in U-251 and E-98 cells 3 days after their transfection with either non-targeting (NT) or β1 integrin siRNA based on flow cytometry (A) or confocal microscopy on laminin 511 coated culture surface (B). Red nuclei, all cells; green fluorescence, cells retaining β1 integrin expression despite siRNA treatment. (C) Number of migrated U-251 and E-98 cells per spheroid after transfection with NT or anti-β1 integrin siRNAs from spheroids on laminin 511 coated culture surface (24 h), in media with isotypic IgG1 and control cRADfV peptide or adhesion-perturbing anti β1 integrin 4B4 mAb combined with cRGDfV peptide (related to Fig. 5B). Data represent 16-32 (U-251) and 15-24 (E-98) spheroids per condition from 3 independent siRNA transfections. Values display the median (black line), 25/75 percentiles (boxes) and maximum/minimum (whiskers). P-values shown were obtained using the Mann-Whitney test. Scale bar, 100 μm.

References.

Hughes, C. S., Postovit, L. M. and Lajoie, G. A. (2010). Matrigel : A complex protein mixture required for optimal growth of cell culture. *Proteomics* **10**, 1886–1890.